ANTI GENOTOXIC ACTIVITY OF ANDROGRAPHIS PANICULATA AGAINST CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY IN SWISS ALBINO MICE.

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Abstract:
The present study was designed to investigate the effect of *Andrographis paniculata* (*A. paniculata*) on Cyclophosphamide (CP) induced genotoxicity in mice. A dose of 4000mg/kg *A. paniculata* was selected for the study. Animals received 14days pretreatment of *A. paniculata* followed by induction of genotoxicity by CP (40mg/kg) 24 hours before sacrifice. Mice bone marrow micronucleus assay were employed for the study. Results shows the CP induced significantly increased bone micronucleus Polychromatic, Micronucles, Bilobed cells and P/N ratio, however CP decreased Normal cells. Treatment with *A. paniculata* Results showed that a significant increase normal cells, Decreased in Micronuclei (MN) formation in polychromatic erythrocytes of Micronucles, Bilobed cells and P/N ratio. Micronuclei (MN) formation in polychromatic erythrocytes, produced cytotoxicity in mouse bone marrow cells Pretreatments with *A. paniculata* , significantly inhibited cytotoxicity in mouse bone marrow cells induced by CP. Our studies revealed that *A. paniculata* has protective effect against genotoxicity induced by CP.

Keywords: CP**,** Micronuclei, *A. paniculata*, P/N ratio.

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Introduction

Genotoxicity is destructive effects on a genetic material affecting its integrity and fidelity by genotoxins which are known as mutagens they can cause mutations. Genotoxins include both physical (UV radiation, abnormal temperatures) and chemical genotoxins (alkylating agents, heavy metals, toxins, etc). The term damage applies not only to mutations but also to spontaneous mutations due to DNA lesions that originate endogenously. A significant fraction of DNA damage involves the modification or elimination of bases, without alteration of sugar-phosphate backbone, so that DNA coding capacity is highly abolished at the lesion site (Dev S., 1999).

Cyclophosphamide (CP) is a widely used antitumor prodrug that is effective against a broad spectrum of human cancers including breast cancer and lymphomas but it is toxic to the normal cells. The toxicity outline is characterized by myelosuppression and urotoxicity (Dollery C., 1999). Activation of CP to 4-hydroxycyclophosphamide is catalyzed by the hepatic cytochrome P450 (CYP) isozymes CYP2B6, 2C9 and 3A4 (with 2A6, 2C8 and 2C19 making more minor contributions). Competing with C-4 hydroxylation of CP is a minor (~10%) oxidative pathway that leads to N-dechloroethylation and the formation of the neurotoxic chloroacetaldehyde. CYP3A4 is primarily responsible for this undesirable side-chain oxidation with a minor contribution from CYP2B6 (Boyd V.L et al., 1986)

A. paniculata (AP) is an important medicinal plant and widely used around the world. It belongs to the family Acanthaceae. AP is used as a traditional herbal medicine in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand (S. Akbar 2011, M. H. Kabir 2014) and is ethnobotanically used for the treatment of snake bite, bug bite, diabetes, dysentery, fever, and malaria (I. H. Burkill1996).the plant contain flavonoids, Phytosterols, diterpenoids, Bitter glycosides, Phenols and Xanthones (S. Akbar 2011, M. H. Kabir 2014, K. Jarukamjorn and N. Nemoto 2008, Damu AG1998, Koteswara Rao Y2004, C. Xu,2012, C. Boopathi2000)Plant have reported serval activities such as Herbal extracts of A. paniculata are useful as anti-inflammatory (Sheeja, Shihab, & Kuttan, 2006), antioxidant (Tripathi & Kamat, 2007), antiviral (Calabrese et al., 2000), anticancer (Ajaya et al., 2004), antimicrobial (Singha, Roy, & Dey, 2003), antimalarial (Siti Najila et al., 2002), and are hepatoprotective (Trivedi, Rawal, & Patel, 2007) agents. It had also shown immunostimulatory (See, Mason & Roshan, 2002), phagocytotic (Matsuda et al., 1994), anti-diabetic (Reyes et al., 2006) and wound healing (Syed Talha Pasha, Roshan. S 2021) activities. However, there is no data available regarding Cyclophosphamide induced genotoxicity from *A. paniculata* extracts, hence the present study was undertaken in mice to evaluate the Cyclophosphamide induced genotoxicity from ethonolic extracts of *A. paniculata*.

Materials and Methods

Chemicals

Cyclophosphamide, Colchicine, Bovine Serum Albumin, Sodium azide are purchased from Sigma-Aldrich Co. Giemsa Stain and May-Gruenwald are purchased from Hi-media. All other chemicals were AR grade and commercially available.

Plant material

The Ethanolic extract of *A.paniculata* batch No-210316/01 is gift sample obtained from Himalaya drugs, Bangalore, India.

Extracts was performed a phytochemical screening and evaluation of anti genotoxic activities.

Animals

Swiss albino mice weighing 25 ± 5 g, 6-7 weeks old were obtained from the central animal house of H. S. K. College of Pharmacy and Research Centre, Bagalkot. The animals were housed at room temperature (22-28 ºC) with 65 ± 10% relative humidity for 12 hr dark and light cycle and given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum.* The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (HSKCP/IAEC, Clear/1/2020-12/R&D91).

Experimental design

Twenty four swiss albino mice either sex were housed in 4 groups (n=6).

Group –I Control as distilled water and served as negative/vehicle control.

Group –II CP (40 mg/kg of b.w.) was administered intraperitoneally (i.p) on 30th day and sacrificed after 24hr

Group-III AP extract at 400mg/kg of body weight (b.w) for 30 days post orally (p.o) and CP (40 mg/kg of b.w.) was administered intraperitoneally (i.p) on 30th day .

on 30th day and sacrificed after 24hr of CP administration with chloroform anesthesia. All groups sacrifice (Mohamed A. et al., 2011)

Bone marrow micronucleus assay and Chromosomal aberration assay

Bone marrow micronucleus test was carried out according to the method of Schmid (1975). The experimental animals were sacrificed by anesthetized. Femur and tibia were removed from the animals for collection of bone marrow, each leg was used for individual assay. About 0.5-1 ml of the bovine albumin medium was inserted at the lower epiphysial end. The marrow was collected from and suspension was subjected to centrifugation at 1000 rpm for 8 min. The obtained pellet was suspended with few drops of fresh serum and slides were prepared and air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 minutes and stained with May-Grunwald/Giemsa at pH 6.8. The slides were scanned under oil immersion (100X) in microscope for the presence of MN in bone marrow cells by counting a total of about 200 erythrocytes per animal (D'Souza U.J.A et al., 2002).

The method used for obtaining chromosome preparations from the bone marrow cells was based on the technique used by Yosida and Amans (1965) with some modifications. Bone marrow was collected with Hank's balanced salt solution (HBSS), centrifuged at 800 rpm for 10 min. pellet was suspended with hypotonic solution of potassium chloride (75mM Kcl) and incubated at 37^0c for 10 min. suspension was centrifuged and discarded the supernatant, cells were fixed in fixative solution (3 parts Ethanol: 1 part Glacial acetic acid) for 10 min at room temperature, then centrifuged at 4^0c for 10 min and the supernatant was discarded. Fixation was repeated twice and allowed the tubes to stand for 30 min or overnight at 4^0c. Fixation was repeated for overnight stored cells prior to the preparation of slides. Using a pasture pipette, 3 to 4 drops of the suspension from the height of 90 cm was dropped onto a microscope slide, which was previously soaked in water and methanol 3:1 ratio and precooled to 4^0c. Prepared slides were dried and stained with 10% Geimsa stain solution and analysed 50 metaphases per animal.

Statistical analysis

The results obtained from the pharmacological experiments were statistically analyzed using SPSS Version 19.0. The data is presented as mean of ± SEM. *a $p<0.001$, *b $p<0.01$ and *c $p<0.05$. The results were expressed as MEAN + SEM, n=6. The value of $p<0.05$ considered as significant. Statistical comparison was performed by one-way ANOVA followed by Tukey's post-test.

Results

Anti-genotoxic effects of EAP **extract against CP induced Micronuclei in mice**

The present study revealed the potent antigenotoxic effect EAP extracts by Micronuclei assay in swiss albino mice. In CP induced control group animals showed significantly reduced the number of normal cells *($p<0.001$)* as compared to normal group. In contrast EAP +CP treated groups showed increase in normal cells *($p<0.001$)* as compared to control groups. Similarly EAP an alone treated groups does not show any significant reduction of normal cells as compared to normal group.

Similarly, the number of abnormal cells such as (polychromatic, micronuclei, bilobed) were significantly (*$p<0.001$)* increased by CP exposed control group animals as compared to normal group. Where as in EAP +CP treated groups showed decreased polychromatic cells (*$p<0.05$ to $p<0.001$)*, micronuclei cells (*$p<0.01$ to $p<0.001$)*, bilobed *($p<0.001$)* as compared to control groups. Results are presented in (Table:1 and figure 1)

Table: 1 Anti-genotoxic effects of *A. Paniculata* on bone marrow cells against CP induced Micronuclei assay.

Groups	No of normal cells (N)	No of polychromatic cells (P)	No of micronuclei cells	No of bilobed cells	P/N Ratio
Normal	196.2± 2.457	5.789 ± 1.016	5.176 ± 0.8743	5.677± 1.334	0.08±0.004
CP (50mg/kg) 24hrs	75.17± 15.14[a]	55 ± 5.203[a]	34.79 ± 7.427[a]	32.97± 2.401[a]	0.99±0.02354[a]
EAP 400	172 ± 4.626[ns]	7.17±1.194[ns]	8.6±1.232[ns]	7.66±3.377[ns]	0.21±0.0245[ns]
EAP400 + CP (50mg/kg)	154.7±1.764***	31.67±1.732***	14.5±2.253*	8.175±3.301***	0.34±0.015***

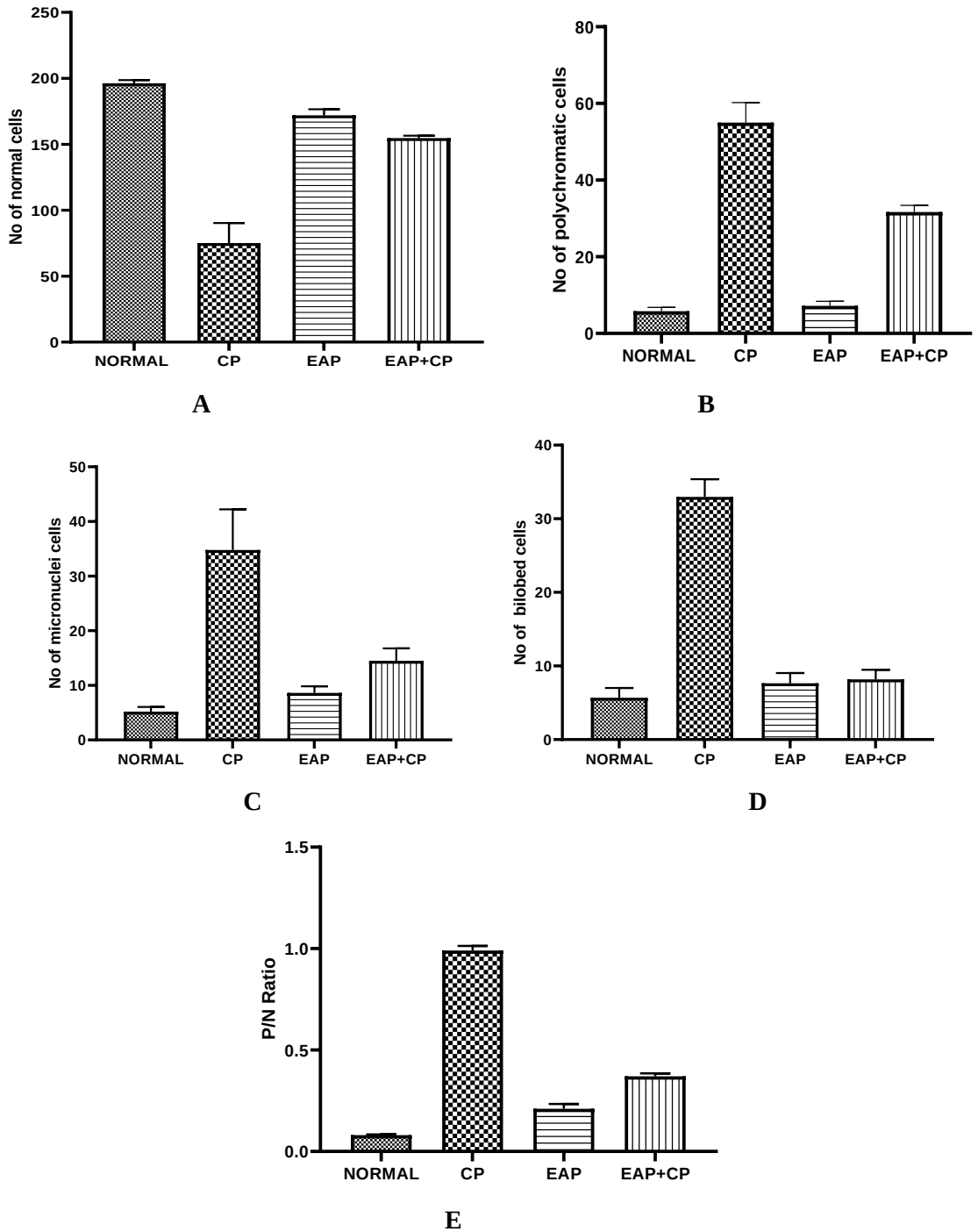

Figure1 : Showing the (A)Number of normal cells (B) Number of Polychromatic (C) Number of Micronuclei (D) Number of bilobed and (E) P/N ratio in bone marrow cells against CP induced Micronuclei assay.

Discussion

DNA damage is frequent occurrence in cells exposed to oxidative stress. The ROS are DNA damaging agents, which produce a series of DNA lesions, including base damage, single or double strand breaks, as well as DNA–DNA or DNA–protein crosslinks. Double strand breaks are the most important consequence of oxidative stress since they lead to cell death. Cyclophosphamide is known to cause DNA strand breakage by apoptosis initiated by ROS, modulation of cell cycle and other antiproliferative effects. Thus, it can damage DNA during any phase of cell cycle, and therefore, it is not phase specific. The main mechanism is inhibition of DNA replication, as the interlinked strands cannot separate leading to cell death (Souria M D. et al., 2010). Extent of DNA damage was evaluated by Chromosomal aberration assay, cyclophosphamide is an anticancer agent, in presence of cytochrome P450 enzyme CP is

metabolically activated to produce reactive oxygen species such as phospharamide and acrolein these formed ROS can cause damage to DNA at various cell cycle, at metaphase cyclophosphamide induces DNA damage by formation chromatid break, chromosomal ring, chromosomal fragments, chromosomal gaps, chromosomal association (Saxena A.K. and Sing G.,1998).

Present study animals treated with CP showed marked increase in no of chromosomal ring, chromosomal fragments, chromosomal gaps and chromosomal association as compared to normal. The MCS showed the significant reduction of chromosomal aberrations as compared to control group animals. The antigenotoxicity effect of extracts against CP may be due to its potent antioxidant effect by inhibiting the formation of ROS and DNA damage. Micronuclei formation is due to delay in embryonic progression was reported following parental exposure to irradiation, other alkylating chemicals and pesticides. The proportion of cells with micronuclei is the same from the 2 to 8 cell stage; it indicates all micronuclei are formed during the first cell division. Since no new micronuclei are generated in the next two cleavage divisions, the average number of micronuclei per cell decreases as the cell number increases. This suggests that replicated micronuclei segregate randomly into the daughter cells during mitosis, as plasmids do in bacteria. Thus, genetic material is lost to the embryo (Broers J.L. et al., 1997; Austin S.M. et al., 1994; Mozdarani H. and Nazari E., 2009). The animals treated with CP caused the DNA damage and induced the micronuclei formation by affecting the first zygotic division were very similar to the main nuclei: During interphase, the micro nuclear chromatin was decondensed and round with a single nucleolus, while during mitosis the micronuclear chromatin was condensed. In cleavage stage embryos, the micronuclei followed the same transient nuclear volume change exerted on the main nucleus, suggesting that micronuclei are in communication with the main nucleus and cytoplasm within the cell. (Tian Y. and Yamauchi T., 2003; Lisanne G. and Bernard R.P., 2011).

Present study, animals treated with CP significantly induced the micronuclei formation and this is may be due to inhibiting the first cell division and causing chromatin decondensation round with a single nucleolus, while during mitosis the micronuclear chromatin was condensed. In cleavage stage cell, the micronuclei followed the same transient nuclear volume change exerted on the main nucleus, this result in increased no of micronuclei with other abnormal cell formation such as polychromatic and bilobed, when animals treated with extract EAP showed significant reduction in micronuclei, polychromatic and bilobed which are induced by cyclophosphamide.

The main constituents of extracts are *A. paniculata* extract showed the presence of several bioactive molecules such as flavonoids and di terpenoids andrographolides, β- sitosterol, stigmasterols,4-ketopinoresinol and α- Amyrin (Koteswara, Vimalamma, Venkata Rao, & Tzeng, 2004). bioactive phenolic compounds such as coumarins, phenylpropanoids, flavones like apigenin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside; flavonols like quercetin, rutin and flavanones and catechins, which has been reported having potential antioxidant activity against oxidative stress. In our present study we observed antigenotoxic activity such as micronuclei, chromosomal aberration assay activity may be due to presence of active constituents like flavonoids, flavanols flavanones, coumarins catechins in EAP extract which confirms potential antispermatogonial activity by its antioxidant properties In conclusion, present study suggests a potential role of methanol extract of *A. paniculata* against Cyclophosphamide induced genotoxicity. Further studies are required to explain the cellular mechanism to understand the exact mechanism and actions.

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CONFLICT OF INTEREST

We have no conflict of interest to declare

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